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CYTOLOGICAL AND BIOCHEMICAL STUDY OF CO-OCCURING DIPLOID, TETRAPLOID AND HEXAPLOID INDIVIDUALS OF *SETARIA VERTICILLATA* (L.) BEAUV. (POACEAE)

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ABSTRACT

Three nearly distinct morphotypes with chromosome numbers of $n = 9$, 18 and 27 were encountered in *Setaria verticillata* (L.) Beauv., collected from the plains of Punjab, India. Morphological and cytological observations were supplemented with data from biochemical studies to assess genetic divergence and to understand the relationships between morphotypes. The three cytotypes exhibited constant variations in several biochemical characters. Some of these were directly related to ploidy level. The cytological behaviour of tetraploids parallels that of an allotetraploid, while that of hexaploids compares well with that of an autoallopolyploid. These observations were further substantiated with information from phenolic compounds and albumin patterns. Phenolic compounds, albumin, globulin and isozyme patterns were highly specific within cytotypes.

KEY WORDS: Angiosperms, Poaceae, *Setaria verticillata*, chemotaxonomy, soluble proteins, free amino acids, starch content, nucleic acids, ascorbic acid, lipids, phenolics, albumins, globulins, isozymes.

Setaria verticillata (L.) Beauv., a member of the tribe Paniceae of the Poaceae, is widely distributed in a variety of habitats on the plains of northwest India. The present study from the plains of Punjab, revealed the presence of three distinct morphological forms which were propagated by seed in the botanical garden under uniform nursery conditions. The garden grown plants compared well to those of the natural populations, suggesting genetic differentiation between the populations. Cytological analysis of the morphotypes growing in nature, as well as those maintained in the botanical garden, showed

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them to be cytotypes at diploid, tetraploid and hexaploid levels with $n = 9$, 18 and 27 respectively (Figure 1). It was considered desirable to supplement the observations on morphological and cytological characters, with data from biochemical studies, in order to assess the degree of genetic divergence and to highlight interrelationships between these taxa.

Material and Methods

The plants for the present study were collected from areas in and around seven major localities on the plains of Punjab (Table 1, Figure 2). Chromosome numbers were ascertained from young inflorescences by the usual acetocarmine technique. Voucher specimens are preserved in the Herbarium, Punjab Agricultural University, Ludhiana (PAU). The diploid, tetraploid and hexaploid taxa did not show any preference for a particular type of habitat but rather existed in a haphazard manner. The relative proportion of the prevailing cytotypes at the investigated sites is presented in Table 1. The morphological features marking these cytotypes from each of the seven localities were maintained under uniform nursery conditions in the botanical garden. A preliminary survey of biochemical characteristics suggested that differentiation in biochemical features paralleled that in morphological features. Ten plants of each cytotype were collected at random from garden grown plants for biochemical analysis. Where leaves were utilized for such studies, they were collected from the tip of erect culms just at the time of emergence of the inflorescence.

Total soluble proteins, starch and soluble sugars were estimated by the methods of Lowry, *et al.* (1951), McCready, *et al.* (1950), Loewus (1952 [for total soluble sugars]) and Shallenberger & Moores (1957 [for chromatographic studies]), respectively. Determination of free amino acids was done by the methods of Lee & Takahashi (1966; [total free amino acid content]) and Consden, *et al.* (1944 [for chromatographic studies]). The estimations of total lipids and ascorbic acid were made by the methods of Folsch, *et al.* (1957) and Aberg (1958) respectively. For the study of phenolic patterns, the method of Frost, *et al.* (1975) was used. The quantitative estimation of nucleic acids (DNA and RNA), was made as per methods of Burton (1956) and Ogur & Rosen (1950), respectively. The method of Davis (1964) was employed for polyacrylamide gel electrophoresis. Staining of proteins, esterase and peroxidase isozymes was done by the method of Weber & Osborne (1969), Tripathi, *et al.* (1983) and Kuhns & Fretz (1978) respectively.

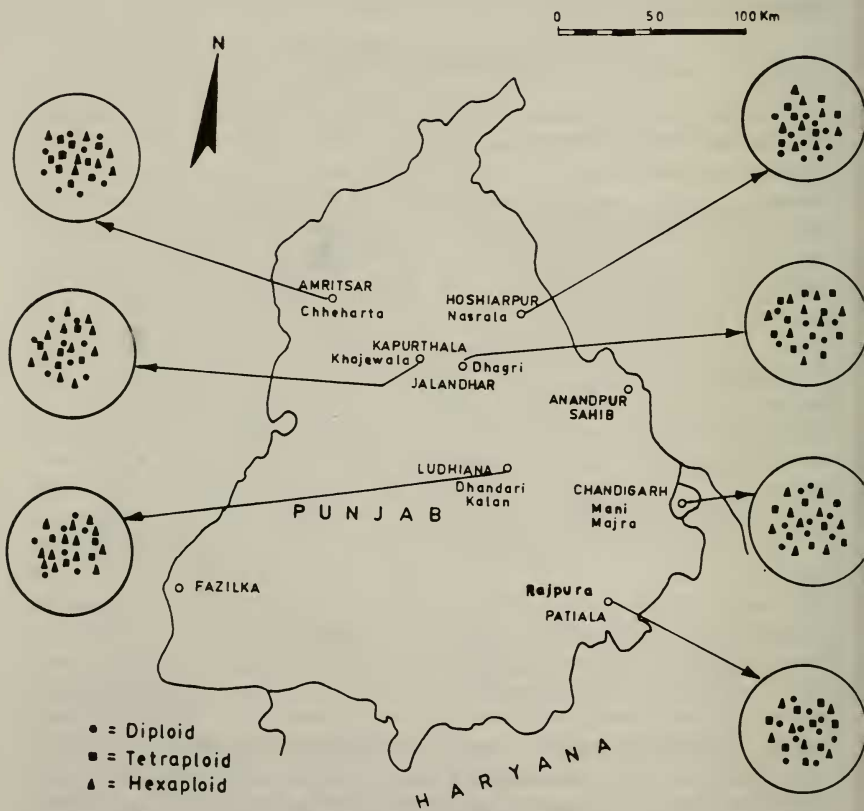

Figure 1. Map showing the relative proportion of 2X, 4X and 6X individuals at each of the seven localities sampled in Punjab.

Table 1. Actual collection sites with vouchers listed. Numbers in parentheses represent sample sizes.

Amritsar Site (Chheharta)

diploids (8)	(4501,4502,4504,4509,4510,4517,4520,4523)
tetraploids (9)	(4503,4505,4506,4507,4514,4515,4521,4522,4524)
hexaploids (7)	(4508,4511,4512,4513,4516,4518,4519)

Kapurthala Site (Khojewala)

diploids (8)	(4553,4557,4558,4559,4562,4563,4569,4570)
tetraploids (5)	(4550,4555,4556,4565,4573)
hexaploids (11)	(4551,4552,4554,4560,4561,4564,4566,4567,4568, 4571, 4572)

Hoshiarpur Site (Nasrala)

diploids (10)	(4527,4528,4529,4532,4533,4539,4542,4543,4545, 4547)
tetraploids (6)	(4530,4534,4535,4537,4541,4546)
hexaploids (8)	(4525,4526,4531,4536,4538,4540,4544,4548)

Jalandhar Site (Dhagri)

diploids (6)	(4575,4579,4582,4586,4588,4598)
tetraploids (10)	(4576,4577,4583,4584,4585,4589,4590,4591,4593, 4594)
hexaploids (8)	(4578,4580,4581,4587,4592,4595,4596,4597)

Ludhiana Site (Dhandari Kalan)

diploids (7)	(4601,4610,4611,4614,4617,4621,4623)
tetraploids (4)	(4600,4605,4609,4612)
hexaploids (13)	(4602,4603,4604,4606,4607,4608,4613,4615,4616, 4618, 4619,4620,4622)

Table 1 continued.

Chandigarh Site (Mani Majra)

diploids (8)	(4627,4628,4629,4632,4636,4637,4641,4646)
tetraploids (7)	(4631,4634,4635,4640,4643,4644,4647)
hexaploids (9)	(4625,4626,4630,4633,4638,4639,4642,4645,4648)

Patiala Site (Rajpura)

diploids (10)	(4650,4651,4653,4659,4660,4662,4668,4669,4670, 4671)
tetraploids (8)	(4652,4654,4655,4656,4665,4666,4667,4673)
hexaploids (6)	(4657,4658,4661,4663,4664,4671)

Results and Discussion

The course of meiosis was perfectly normal in diploid and tetraploid forms. Meiotic irregularities of high order were found in the hexaploid taxa. During Metaphase I (M_I) of the hexaploids, univalents and multivalents were found, along with bivalents in some pollen mother cells (PMCs). Laggards were also recorded at Anaphase I (A_I). These aberrations affected the pollen fertility adversely to the degree where only 78% of the pollen grains were determined to be normal. Gupta & Singh (1977), who examined eighteen species of this genus, did not discover diploid taxa in this species. Plant height was observed to increase with increase in ploidy level (Table 2). Increase in internodal length contributed significantly to this increase in plant height. Internodal colour, coupled with plant height proved useful in the identification of cytotypes in the field (Table 2). Of the many leaf characteristics and inflorescence traits that were observed to be directly related with ploidy level, stomatal and pollen grain size proved highly discriminatory (Table 2). Stomatal frequency per unit area, however, decreased with progressively higher levels of ploidy (Table 2). Similar observations were earlier recorded by Sachdeva & Bhatia (1979) and Sachdeva & Kals (1981) in *Cynodon dactylon* and *Dactyloctenium aegyptium* respectively. So much is the constancy and reliability of these characters that they can unambiguously be employed to identify the cytotypes, even when spikes are not at a favourable stage for undertaking meiotic studies.

Biochemical evaluation of the cytotypes revealed considerable intraspecific variation. Variations in DNA, RNA, ascorbic acid, lipids and total soluble proteins were observed to be related to ploidy level (Table 3). Whereas amounts

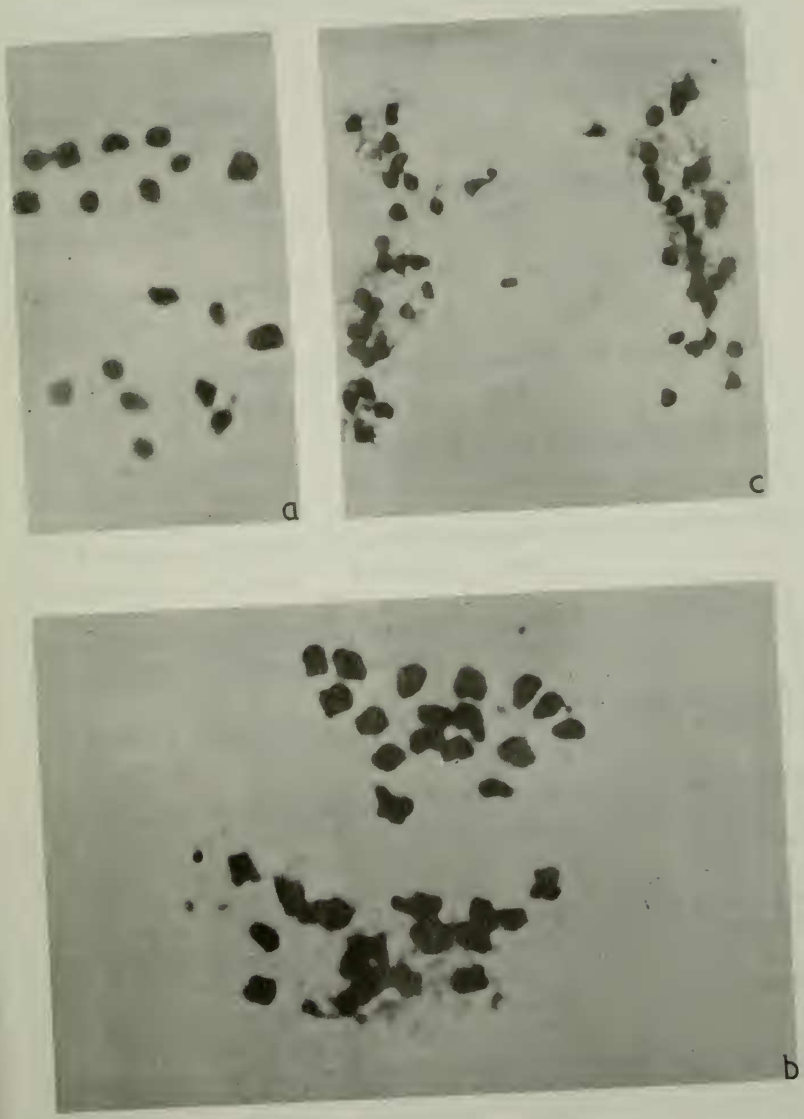

Figure 2. Photomicrographs of meiotic stages of the three cytotypes (all X 1500); a. $n = 9$ at late Anaphase I; b. $n = 18$ at Metaphase II; c. $n = 27$ at Anaphase I.

Table 2. Comparison of some morphological characters in three cytotypes of *Setaria verticillata* (L.) Beauv. ± denotes standard deviation.

Characters		Diploid (n=9)	Tetraploid (n=18)	Hexaploid (n=27)
Vegetative Traits				
Plant height (cm)	Range	12.0-28.0	35.0-60.0	62.0-90.5
	Mean	18.0±3.4	45.2±9.9	78.0±9.2
Internode length (cm)	Range	1.5-3.5	5.5-7.0	6.5-9.0
	Mean	2.6±0.7	6.2±0.7	7.9±1.0
Internode colour		greenish yellow	light green	deep green
Number of leaves per culm	Range	8.0-16.0	8.0-16.0	12.0-17.0
	Mean	14.2±2.5	14.5±2.1	15.0±1.5
Leaf blade length (cm)	Range	6.6-9.2	7.4-15.0	14.0-22.0
	Mean	7.9±1.0	12.5±2.9	17.9±2.8
Leaf blade width (cm)	Range	8.5-15.0	10.0-18.0	14.5-21.9
	Mean	10.5±1.0	13.9±1.8	17.5±2.5
Leaf sheath length (cm)	Range	3.5-4.3	6.3-14.2	6.5-10.0
	Mean	4.4±0.5	10.3±0.7	8.0±0.2
Stomatal frequency (per mm^2)	Mean	52.9±2.2	41.2±1.7	32.9±1.5
Stomata size (μm)	Mean	23.0 x 22.6	26.9 x 25.6	35.2 x 27.8
Floral traits				
Number of spikes per inflorescence	Range	3.0-5.0	4.0-7.0	6.0-8.0
	Mean	3.8±1.5	4.9±0.9	7.2±0.8
Spike length (cm)	Range	2.2-4.4	6.6-9.2	10.3-14.8
	Mean	3.5±0.5	7.9±1.0	12.5±0.7
Spikelet length (mm)	Range	1.1-1.2	1.5-1.9	2.1-2.5
	Mean	0.9±0.1	1.7±0.2	2.2±0.2
Fertile pollen size (μm)	Mean	26.4 x 25.1	34.2 x 32.7	47.2 x 45.0
Pollen fertility	Mean	100%	100%	78%

Table 3. Comparison of different biochemical parameters in three cytotypes of *Setaria verticillata* (L.) Beauv. Values shown are mean ± standard error.

Characters	Diploid (n=9)	Tetraploid (n=18)	Hexaploid (n=27)
Total soluble protein (mg protein/g leaf fresh weight)	6.44±0.06	5.52±0.05	4.84±0.01
Total free amino acid (mg amino acid/g leaf dry weight)	14.4±0.08	13.1±0.02	14.8±0.04
Starch content (mg starch/g leaf dry weight)	81.6±0.32	87.0±0.32	49.5±0.68
Total soluble sugar (mg sugar/g leaf dry weight)	4.6±0.12	3.3±0.08	3.31±0.15
RNA (mg RNA/g leaf dry weight)	6.43±0.17	10.59±0.08	11.19±0.14
DNA (mg DNA/g leaf dry weight)	2.62±0.01	5.19±0.14	9.56±0.09
Ascorbic acid (mg ascorbic acid/100 g leaf fresh weight)	30.8±1.4	35.3±0.9	40.5±1.7
Total lipids (%)	5.9	6.5	7.2

of DNA, RNA, ascorbic acid and lipids increased with increase in ploidy level, the soluble protein content registered a continuous decrease with increase in chromosome number (Table 3). Kumar (1987) had observed a similar increase in DNA and RNA contents that was directly related with ploidy level in *Cynodon dactylon*. However, he reported no such linear relationship in soluble proteins. Starch content was observed to be highest in the tetraploids, followed by diploids and hexaploids (Table 3). Considerable quantitative variations in free amino acids were noticed. The diploids were quite rich in γ - amino butyric acid and β - alanine. The tetraploids revealed much higher levels of cysteic acid and cysteine. The hexaploids exhibited greater amounts of aspartic acid, glutamic acid and glutamine, glycine and serine, alanine, proline, valine and methionine, and phenylalanine (Table 4).

The three cytotypes also varied considerably in sucrose, glucose and fructose content. As invertase is known to catalyze the hydrolysis of sucrose to

Table 4. Amino acid composition (mg amino acid/g dry leaf weight) in three cytotypes of *Setaria verticillata* (L.) Beauv.

Characters	Diploid	Tetraploid	Hexaploid
	(n=9)	(n=18)	(n=27)
Cysteic acid + Cysteine	0.32	0.54	0.40
Aspartic acid	0.78	0.80	0.86
Glutamic acid + Glutamine	1.20	1.28	1.40
γ-Amino butyric acid	0.85	0.20	0.20
β-Alanine	0.75	0.40	0.40
Glycine + Serine	1.17	1.15	1.26
Asparagine	0.57	0.35	0.40
Threonine	0.29	0.40	0.20
Alanine	0.56	0.90	1.06
Tyrosine	0.48	0.39	0.40
Hydroxyproline	0.06	0.03	0.02
Histidine	-	-	-
Lysine	-	-	-
Arginine	0.37	0.37	0.30
Proline	0.26	0.57	0.71
Valine	0.67	0.78	0.91
Tyrosine	-	-	-
Phenylalanine	0.54	0.54	0.62
Leucine + Isoleucine	0.95	0.98	1.00

Table 5. Sucrose, glucose and fructose contents, and invertase activity in three cytotypes of *Setaria verticillata* (L.) Beauv.

Characters	Diploid (n=9)	Tetraploid (n=18)	Hexaploid (n=27)
Sucrose (mg sucrose/g dry leaf weight)	2.12	1.42	2.37
Glucose (mg glucose/g dry leaf weight)	6.51	4.03	7.37
Fructose (mg fructose/g dry leaf weight)	3.51	8.03	3.42
Invertase activity (μg of glucose + fructose produced/mg of protein/hr)	112	183	125

hexoses, and play a key role in growth by controlling sucrose storage and utilization (Ricardo & Rees 1970; MacLachlan, *et al.* 1970; Shukla, *et al.* 1973), the activity of this enzyme was also assayed. The activity was observed to be much higher in hexaploids which exhibited comparatively much higher combined levels of glucose and fructose (Table 5).

Phenolic compound chromatographic patterns were observed to be of great assistance in identifying the different cytotypes. Although identification of compounds is considered significant in studies such as this, it was not possible to identify the compounds producing the spots on the chromatograms. However, much useful information can be gathered from unidentified chromatographic spots (Alston & Turner 1963; Grant 1968; Dass, *et al.* 1976). Twelve spots were observed in diploid cytotypes. Tetraploids and hexaploids exhibited fourteen spots each. Although as many as nine spots were common to all three cytological races, each of the cytotypes exhibited cytotype specific spots (Figure 3). Spots 11 and 12 were confined to diploids only. Spots 13 and 14 were present only in tetraploids. Hexaploids were distinguishable from others by the cytotype specific spot 18. Spot number 10 was found only in diploids and hexaploids, whereas tetraploids and hexaploids were observed to share spot numbers 15, 16 and 17.

Electrophoretic banding patterns of albumins and globulins showed that these proteins could unambiguously be employed for identification of cytotypes (Figure 4). Most of the albumin bands depicted by diploids were also observed in tetraploids. The hexaploids, apart from containing albumin bands from diploids and tetraploids, revealed a characteristic band in zone D. Globulin patterns likewise were cytotype specific. The peroxidase and esterase isozyme

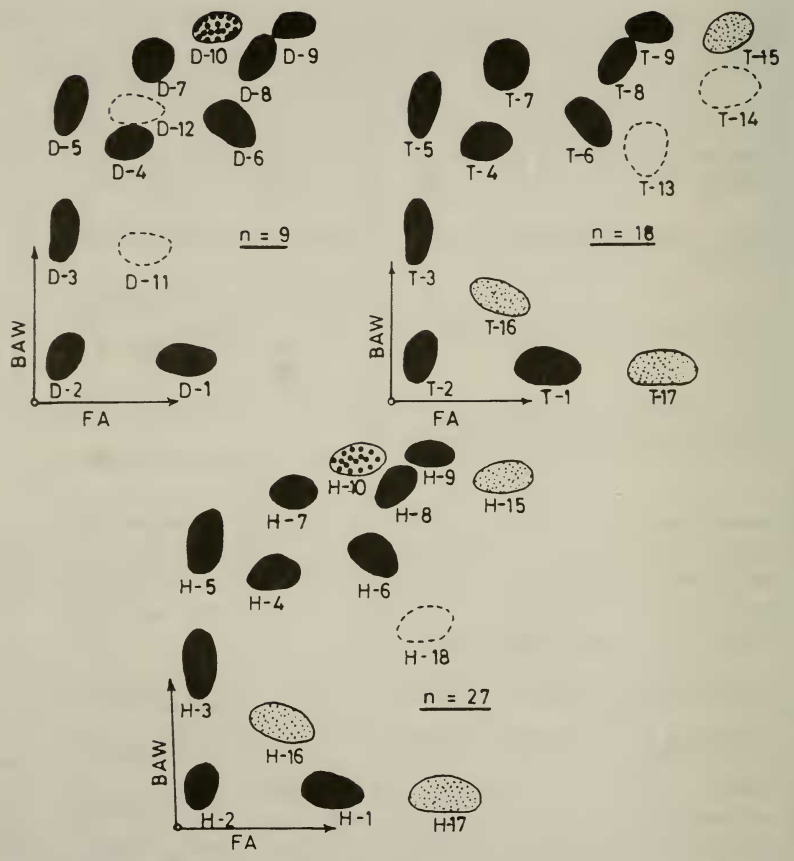

Figure 3. Chromatographic patterns of phenolic molecules in diploid (n = 9), tetraploid (n = 18) and hexaploid (n = 27) taxa. Spots with broken lines indicate the characteristic spots of the cytotypes. Color of spots, uv/uv + NH_3: D-1, T-1, H-1 - Y/FY; D-2, T-2, H-2 - GY/GY; D-3, T-3, H-3 - LBlW/LYG; D-4, T-4, H-4 - B/BY; D-5, T-5, H-5 - P/P; D-6, T-6, H-6 - LY/FY; D-7, T-7, H-7 - Y/GY; D-8, T-8, H-8 - Y/Y; D-9, T-9, H-9 - V/FV; D-10, H-10 - GBl/DuBl; D-11 - YG/YG; D-12 - LY/YG; T-13 - Bl/FBl; T-14 - P/P; T-15, H-15 - LY/FY; T-16, H-16 - LBl/FBl; T-17, H-17 - Y/Y; H-18 - Y/Y. F = Fluorescent; L = Light; Y = Yellow; G = Green; Bl = Blue; W = White; B = Brown; P = Purple; Du = Dull; V = Violet.

patterns were also highly taxon specific (Figure 5). The differences observed in peroxidase and esterase paralleled differences previously observed in cultivars of *Zea mays* L. (Cardy & Kannenberg 1982), *Cenchrus* (Nicholson, *et al.* 1985) and *Agropyron junceum* (Moustakas, *et al.* 1986).

Taken together, the cytological behaviour, phenolic patterns, and electrophoretic profiles of albumin and globulin revealed some interesting correlations. Tetraploids invariably exhibited bivalents and absence of quadrivalents. This suggests that this cytotype is not an autotetraploid or segmental allotetraploid. The meiotic behaviour of hexaploids resembles that of an autoallopolyploid. These observations are further substantiated by data derived from phenolic compound and albumin patterns. Phenolic chromatographs and albumin patterns in diploids and tetraploids are different, conforming to the likely alloploid nature (Murray & Williams 1976) of the tetraploids. Hexaploids reveal a sum total of patterns exhibited by diploids and tetraploids, suggesting the possibility that hexaploids in nature might have originated through crosses between unreduced gametes of diploid and tetraploid taxa. However, globulin patterns were not observed to support the idea of hexaploid origin by fusion of unreduced gametes from diploids and tetraploids. Though globulins were taxon specific, they did not show additive patterns. Bala (1988), while working with varieties of *Setaria italica*, noticed that although albumin and globulin patterns were highly discriminatory, they were not especially useful in providing phylogenetic insights. On the other hand, the evolutionary usefulness of cytological and phenolic data is well established. Thus, the hypothesis that hexaploids originated through fusion of unreduced gametes from diploids and tetraploids is not entirely untenable.

The present investigation has revealed considerable morphological, cytological and biochemical variability among the three cytotypes of *Setaria verticillata* examined. How such taxa might be treated so that the presence and nature of the encountered variations is also reflected by their names is not considered here. The concepts involved in addressing this question (that of nomenclature for variants such as those seen in this study) are considered in some detail by Harborne & Turner (1984) in their chapter on the Application of Chemistry at the Intraspecific Level. It seems clear that cytogeographical, chemogeographical and morphogeographical patterns may be exceedingly complex and need not fit neat patterns, nor lend themselves to easily conceived formal nomenclatural units.

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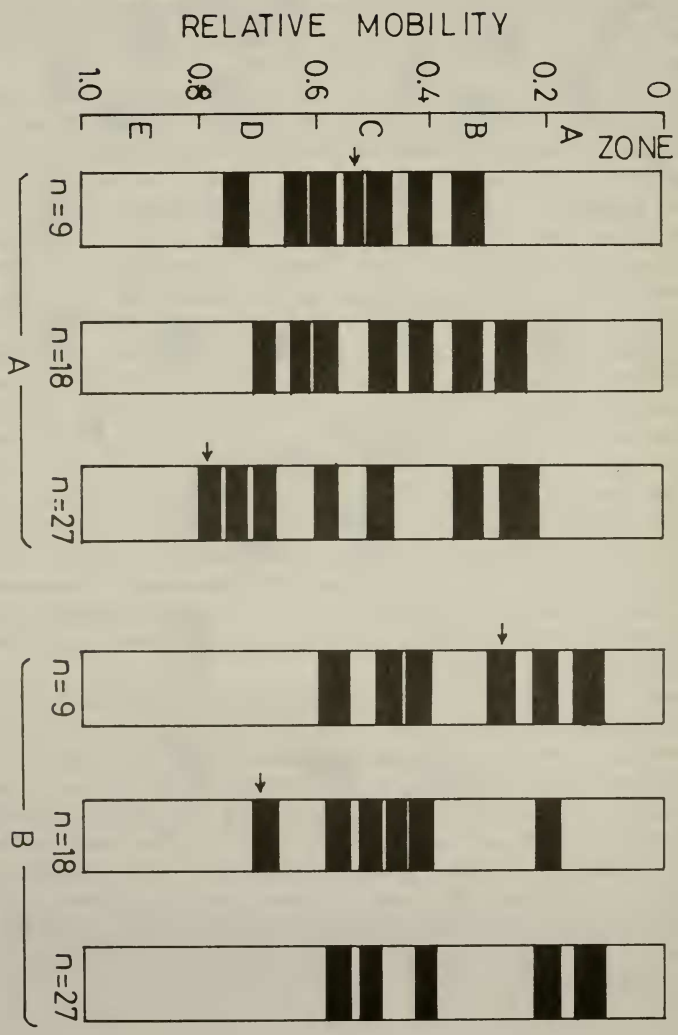

Figure 4. Zymograms of Albumin (A) and Globulin (B) patterns in the three cytotypes.

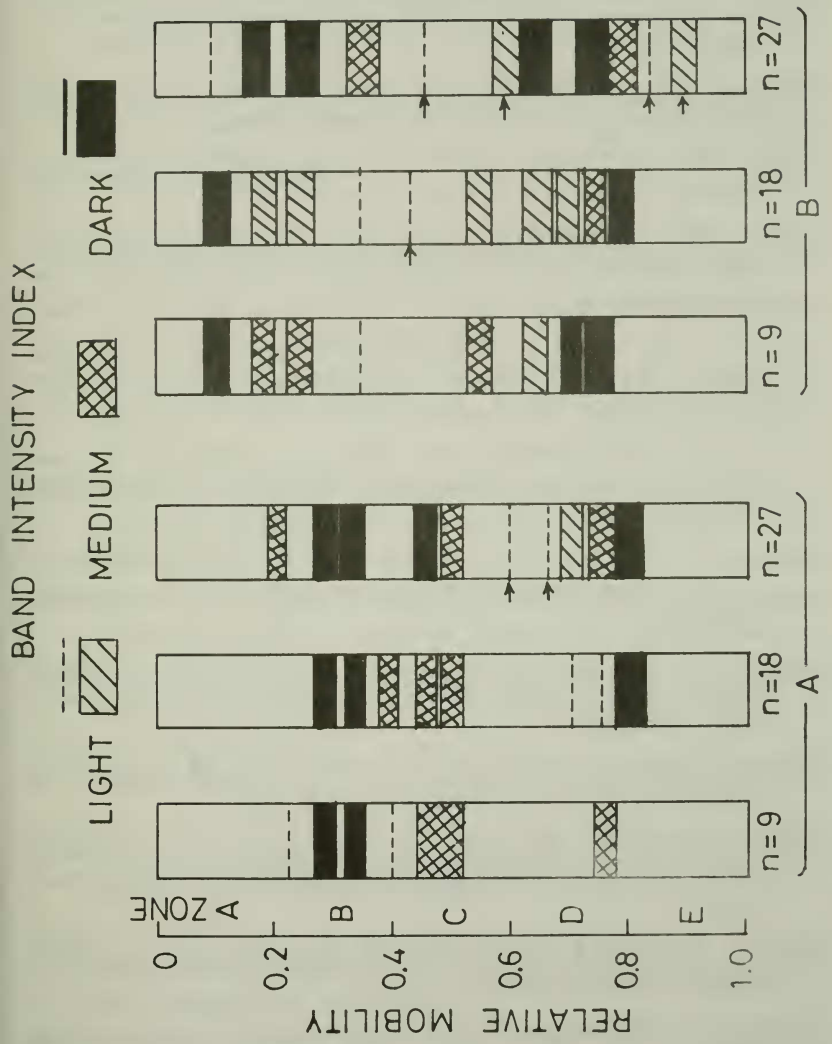

Figure 5. Zymograms of Esterase (A) and Peroxidase (B) isozyme patterns in three cytotypes.

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